CHAPTER 6

STABILITY AND MICROBIAL CONTAMINATION STUDIES.

The determination of stability characteristics of the pharmaceutical dosage forms over extended time periods projects the concept of total quality control to the consumer. Laboratory analysis, manufacturing techniques, and quality control procedures prior to market release attempt to insure product purity, identity, strength and quality at the completion of the manufacturing processes. Stability studies demonstrate that the necessary critical characteristics present at the time of production and release can be expected to be present when the dosage form is administered[1].

Stability of pharmaceutical product is the capability of a particular formulation, in a specific container, to remain within its physical, chemical, microbiological, therapeutic and toxicological specifications. Assurance that the packed product will be stable during its anticipated shelf life must come from an accumulation of the data on the packed drug. These stability data involve selected parameters which, taken together, form the stability profile, 90% of the labelled potency of active ingredient(s) is generally recognised as the minimum acceptable potency level. Expiration dating is the time in which the preparation will remain stable under the recommended conditions of storage.

The prediction of stability of a drug product depends on quantitative mathematical expressions. These expressions permit calculation of degradation rate taking into account the factors such as concentration, temperature and time.

The basic concepts of kinetics and their application in understanding degradation of pharmaceutical systems have been described by several authors[2-7]. Higuchi et al.[8,9] were the first to publish rigorous kinetic studies including reaction and heat of activation. Garrett[10] described a method for prediction of degradation rate from elevated temperature data. While studying degradation of a drug at elevated temperature, the degree of instability of the active ingredient in a stressed preparation is monitored by specific assay procedure. This approach although not very accurate, is quite satisfactory for comparison. The degradation of the drug is expressed as a linear function of time degradation rate is computed.

The assessment procedure for the stability of a pharmaceutical product examines the capability of a particular formulation, in a specific container, to remain within its physical, chemical, therapeutic and toxicological specifications.[11]

The first difficulty arises in attempts to assess the chemical stability of the drug in its complex vehicle, together with the stability of any potentially labile adjuvants. A general methodology for predicting chemical stability uses an accelerated stability test which subjects the material to elevated temperatures and uses the Arrhenius relationship to establish a self life.[12-14] However, in a multiphase system such as a cream, heat may alter the phase distribution and may even crack the emulsion. This

assessment is limited in this type of preparation over a long time at the storage temperature.

6.1. Experimental :

Corticosteroid creams, found satisfactory in in vitro studies, were subjected to stability studies. Triamcinolone acetonide, betamethasone 17-valerate, halcinonide and fluocinolone acetonide formulations were assayed and packed in lacquered aluminium collapsible tubes and stored at air condition temperature (20±2°), room temperature, 37° and 42° at 80% RH for one and half year.

Materials :

Triamcinolone acetonide creams (0.1% w/w)
Betamethasone 17-valerate creams (0.122% w/w)
Halcinonide Creams (0.1% w/w)
Fluocinolone acetonide creams (0.025% w/w)
were formulated and kept on stability.

Methods :

Preparation of standard solutions.

20 mg of pure TA, BV, HAL and FA were weighed accurately and transferred quantitatively into 100 ml amber coloured volumetric flasks separately. They were dissolved and diluted to volume with chloroform. Exactly 10 ml. of above solutions were pipetted out into other 100 ml amber coloured volumetric flasks and then diluted to volume with chloroform. Exactly 10 ml

of above solutions were pipetted out separately into 25 ml amber coloured volumetric flasks.

Preparation of sample solutions.

2 g. of TA, BV and MAL and 8 g of FA Creams equivalent to 2 mg of TA, BV and MAL and 2 mg of FA were weighed accurately and transferred quantitatively into 250 ml dry separatory funnels. 12 ml of water was added and mixed well by shaking. The corticosteroids were extracted with 80 ml of chloroform by shaking for 25 minutes. The chloroform layers were collected in 100 ml dry amber coloured volumetric flask through anhydrous sodium sulphate. Washing was given by 10 ml of chloroform and then diluted to volume with chloroform. The solution was filtered through Whatman filter paper No. 4 and clear filtrate was used for colour development.

Procedure.

Samples were analysed as per the procedure described in Chapter 2, by running standard and sample solutions simultaneously. The absorbance of the sample and the standard solutions were measured at definite wavelength against reagent blank on Beckman Model 35 Spectrophotometer. The content of corticosteroids were calculated by comparison with standard solutions.

TABLE 8-1 : Stability Study on Selected Triamcinolone Acetonide Creams*.

Cream No. 1

Period of Stability	AC		RT		37°		42° 80% RH	
	Assay % w/w	pH	Assay % w/w	pH	Assay % w/w	pH	Assay % w/w	pH
Initial	-	-	0.098	6.3	-	-	-	-
1 Month	0.1	6.2	0.098	6.25	0.102	6.25	0.102	6.3
3 Months	0.102	6.3	0.096	6.3	0.098	6.3	0.098	6.3
6 Months	0.099	6.3	0.098	6.3	0.098	6.3	0.097	6.3
9 Months	0.098	6.3	0.098	6.3	0.102	6.3	0.096	6.3
12 Months	0.1	6.25	0.096	6.3	0.097	6.3	0.098	6.3
18 Months	0.098	6.2	0.098	6.3	0.098	6.3	0.098	6.3

Cream No. 5

Initial	-	-	0.102	6.6	-	-	-	-
1 Month	0.1	6.6	0.102	6.55	0.098	6.65	0.102	6.6
3 Months	0.098	6.6	0.10	6.6	0.098	6.6	0.096	6.6
6 Months	0.096	6.6	0.10	6.6	0.098	6.6	0.094	6.65
9 Months	0.096	6.6	0.098	6.65	0.096	6.6	0.093	6.65
12 Months	0.095	6.6	0.098	6.55	0.097	6.6	0.096	6.5
18 Months	0.096	6.6	0.098	6.6	0.095	6.6	0.098	6.55

Cream No. 11

Initial	-	-	0.105	6.0	-	-	-	-
1 Month	0.102	5.9	0.102	6.0	0.103	6.1	0.103	6.0
3 Months	0.102	5.95	0.102	6.0	0.1	6.1	0.1	6.0
6 Months	0.102	6.0	0.102	5.9	0.102	6.0	0.098	6.0
9 Months	0.099	6.0	0.102	6.0	0.102	6.0	0.099	6.0
12 Months	0.1	6.0	0.1	6.0	0.1	6.0	0.098	6.0
18 Months	0.098	6.0	0.099	6.0	0.1	6.0	0.098	6.0

Cream No. 25

Initial	-	-	0.102	6.4	-	-	-	-
1 Month	0.098	6.3	0.099	6.45	0.098	6.5	0.099	6.6
3 Months	0.095	6.35	0.099	6.5	0.096	6.5	0.102	6.6
6 Months	0.094	6.4	0.099	6.5	0.096	6.5	0.098	6.65
9 Months	0.096	6.4	0.098	6.55	0.095	6.55	Separation	-
12 Months	0.098	6.4	0.098	6.55	0.098	6.6	-	-
18 Months	0.094	6.4	0.097	6.55	Separation		-	-

Cream No. 27

Initial	-	-	0.105	6.6	-	-	-	-
1 Month	0.106	6.6	0.102	6.6	0.104	6.5	0.106	6.6
3 Months	0.105	6.6	0.103	6.6	0.102	6.5	0.105	6.5
6 Months	0.102	6.6	0.102	6.6	0.101	6.55	0.104	6.5
9 Months	0.099	6.6	0.102	6.6	0.102	6.55	0.102	6.45
12 Months	0.099	6.6	0.102	6.6	0.103	6.55	0.101	6.5
18 Months	0.098	6.6	0.102	6.6	0.105	6.55	0.1	6.5

* All the creams (No. 1 to 27) were subjected to stability study but the data on those selected for blanching test only is tabulated.

TABLE 8-2 : Stability Study on Selected Betamethasone 17-Valerate Creams.*

Cream No. 1

Period of Stability	AC		RT		37°		42° 80% RH	
	Assay % w/w	pH	Assay % w/w	pH	Assay % w/w	pH	Assay % w/w	pH
Initial	-	-	0.124	6.6	-	-	-	-
1 Month	0.123	6.5	0.127	6.6	0.123	6.5	0.124	6.7
3 Months	0.121	6.5	0.132	6.6	0.126	6.5	0.123	6.6
6 Months	0.137	6.5	0.137	6.65	0.134	6.6	0.137	6.65
9 Months	0.132	6.6	0.133	6.6	0.132	6.6	0.135	6.8
12 Months	0.131	6.6	0.132	6.6	0.132	6.6	0.137	6.9
18 Months	0.112	6.6	0.123	6.6	0.122	6.6	0.125	6.95

Cream No. 5

Initial	-	-	0.128	7.0	-	-	-	-
1 Month	0.125	6.8	0.123	6.7	0.122	6.8	0.126	6.9
3 Months	0.128	6.8	0.121	6.6	0.126	6.7	0.124	6.95
6 Months	0.127	6.8	0.126	6.6	0.124	6.7	0.125	6.95
9 Months	0.122	6.7	0.123	6.6	0.122	6.7	0.125	6.95
12 Months	0.118	6.75	0.127	6.7	0.127	6.7	0.125	6.95
18 Months	0.140	6.8	0.128	6.6	0.105	6.7	Separation -	

Cream No. 7

Initial	-	-	0.122	6.2	-	-	-	-
1 Month	0.127	6.15	0.120	6.25	0.115	6.2	0.120	6.5
3 Months	0.131	6.15	0.124	6.3	0.131	6.2	0.116	6.7
6 Months	0.126	6.2	0.126	6.35	0.122	6.2	Separation -	
9 Months	0.125	6.2	0.124	6.35	0.123	6.2	-	-
12 Months	0.119	6.2	0.123	6.4	0.121	6.2	-	-
18 Months	0.123	6.2	0.128	6.35	0.128	6.25	-	-

Cream No. 13

Initial	-	-	0.125	6.8	-	-	-	-
1 Month	0.125	6.7	0.127	6.75	0.124	6.8	0.124	6.8
3 Months	0.127	6.7	0.124	6.75	0.126	6.8	0.128	6.8
6 Months	0.128	6.7	0.124	6.7	0.127	6.8	0.128	6.8
9 Months	0.125	6.7	0.125	6.7	0.125	6.75	0.126	-
12 Months	0.121	6.7	0.122	6.7	0.125	6.75	0.124	6.8
18 Months	0.119	6.7	0.121	6.7	0.125	6.75	0.122	6.75

Cream No. 17

Initial	-	-	0.126	7.1	-	-	-	-
1 Month	0.126	7.0	0.124	7.0	0.126	6.8	0.126	6.8
3 Months	0.124	6.9	0.124	7.15	0.125	6.75	0.125	6.85
6 Months	0.124	6.9	0.123	6.9	0.124	6.6	0.124	6.9
9 Months	0.127	6.85	0.123	6.95	0.125	6.95	0.127	6.95
12 Months	0.126	6.85	0.125	7.0	0.127	6.85	0.121	6.95
18 Months	0.123	6.85	0.124	6.95	0.123	6.8	0.119	7.0

* All the creams (No. 1 to 27) were subjected to stability study but the data on those selected for blanching test only is tabulated.

TABLE 8-1 : Stability Study on Selected Halcinonide Creams*

Cream No. 1

Period of Stability	AC		RT		37°		42° 80% RH	
	Assay % w/w	pH	Assay % w/w	pH	Assay % w/w	pH	Assay % w/w	pH
Initial	-	-	0.098	6.4	-	-	-	-
1 Month	0.096	6.3	0.098	6.4	0.097	6.4	0.097	6.4
3 Months	0.097	6.25	0.098	6.35	0.098	6.4	0.098	6.45
6 Months	0.098	6.25	0.096	6.3	0.096	6.4	0.095	6.5
9 Months	0.101	6.2	0.098	6.4	0.094	6.4	0.094	6.5
12 Months	0.101	6.2	0.098	6.4	0.096	6.5	0.095	6.55
18 Months	0.098	6.2	0.102	6.4	0.098	6.5	0.095	6.6

Cream No. 5

Initial	-	-	0.10	6.7	-	-	-	-
1 Month	0.098	6.6	0.10	6.6	0.10	6.45	0.10	6.6
3 Months	0.097	6.65	0.099	6.6	0.096	6.6	0.095	6.6
6 Months	0.10	6.6	0.097	6.5	0.094	6.6	0.094	6.6
9 Months	0.096	6.6	0.096	6.5	0.095	6.5	0.096	6.7
12 Months	0.098	6.6	0.095	6.6	0.095	6.6	0.095	6.8
18 Months	0.098	6.6	0.095	6.6	0.095	6.6	0.094	6.75

Cream No. 11

Initial	-	-	0.101	6.2	-	-	-	-
1 Month	0.104	6.25	0.106	6.2	0.102	6.25	0.102	6.3
3 Months	0.098	6.25	0.097	5.9	0.1	5.95	0.098	5.95
6 Months	0.098	5.92	0.097	6.2	0.097	6.1	0.099	6.1
9 Months	0.098	6.18	0.097	6.1	0.097	6.1	0.099	6.1
12 Months	0.096	6.1	0.097	6.1	0.097	6.12	0.099	6.1
18 Months	0.096	6.0	0.097	6.0	0.098	5.9	0.098	5.9

Cream No. 26

Initial	-	-	0.098	6.4	-	-	-	-
1 Month	0.098	6.5	0.097	6.4	0.095	6.53	0.096	6.5
3 Months	0.1	6.5	0.099	5.9	0.097	6.42	0.101	6.4
6 Months	0.098	6.4	0.1	5.9	0.099	6.15	0.102	6.4
9 Months	0.098	5.8	0.1	6.0	0.098	6.2	0.1	5.7
12 Months	0.098	5.6	0.097	6.0	0.097	5.9	0.099	5.7
18 Months	0.097	5.6	0.097	5.8	0.096	5.8	0.096	5.8

Cream No. 27

Initial	-	-	0.098	6.9	-	-	-	-
1 Month	0.098	6.9	0.097	6.9	0.098	6.85	0.098	6.85
3 Months	0.098	6.8	0.097	6.9	0.1	6.9	0.097	6.9
6 Months	0.098	6.8	0.097	6.9	0.098	6.8	0.096	6.9
9 Months	-	-	0.094	6.9	0.098	6.8	0.097	6.9
12 Months	0.096	6.85	0.097	6.85	0.098	6.8	0.094	6.95
18 Months	0.098	6.9	0.097	6.8	0.097	6.8	0.091	7.0

* All the creams (No. 1 to 27) were subjected to stability study but the data on those selected for blanching test only is tabulated.

TABLE 8-4 : Stability Study on Selected Fluocinolone Acetonide Creams*.

Cream No. 1

Period of Stability	AC		RT		37°		42° 80% RH	
	Assay % w/w	pH	Assay % w/w	pH	Assay % w/w	pH	Assay % w/w	pH
Initial	-	-	0.026	6.4	-	-	-	-
1 Month	0.024	6.4	0.025	6.4	0.019	6.4	0.0185	6.6
3 Months	0.024	6.4	0.023	6.4	0.022	6.4	0.022	6.75
6 Months	0.027	6.3	0.023	6.4	0.022	6.4	0.021	6.85
9 Months	0.024	6.35	0.023	6.4	0.021	6.4	0.023	6.9
12 Months	0.0236	6.3	0.0273	6.4	0.0227	6.4	0.025	7.0
18 Months	0.028	6.3	0.024	6.4	0.024	6.4	Separation -	

Cream No. 5

Initial	-	-	0.0254	6.7	-	-	-	-
1 Month	0.024	6.6	0.023	6.7	0.022	6.5	0.024	6.5
3 Months	0.025	6.6	0.024	6.7	0.024	6.6	0.025	6.5
6 Months	0.021	6.6	0.026	6.7	0.025	6.6	0.021	6.5
9 Months	0.023	6.6	0.023	6.7	0.026	6.6	0.023	6.5
12 Months	0.021	6.65	0.024	6.7	0.025	6.6	0.0215	6.6
18 Months	0.025	6.6	0.024	6.7	0.024	6.6	0.024	6.55

Cream No. 12

Initial	-	-	0.024	6.2	-	-	-	-
1 Month	0.025	6.2	0.024	6.2	0.024	6.25	0.024	6.35
3 Months	0.024	6.2	0.026	6.2	0.026	6.25	0.020	6.65
6 Months	0.024	6.2	0.027	6.2	0.023	6.3	Separation -	
9 Months	0.023	6.25	0.023	6.2	0.024	6.3	-	-
12 Months	0.021	6.25	0.023	6.25	0.025	6.35	-	-
18 Months	0.021	6.3	0.020	6.35	0.020	6.35	-	-

Cream No. 26

Initial	-	-	0.026	6.4	-	-	-	-
1 Month	-	-	0.026	-	-	-	-	-
3 Months	0.023	6.4	0.025	6.4	0.025	6.4	0.026	6.4
6 Months	0.024	6.3	0.025	6.4	0.024	6.4	0.025	6.4
9 Months	0.026	6.25	0.025	6.4	0.023	6.25	0.023	6.45
12 Months	0.026	6.3	0.025	6.4	0.023	6.2	0.021	6.65
18 Months	0.0265	6.25	0.025	6.35	0.022	6.2	Separation -	

Cream No. 27

Initial	-	-	0.025	7.15	-	-	-	-
1 Month	0.024	7.1	0.024	7.1	0.024	7.1	0.025	7.15
3 Months	0.027	7.0	0.024	7.1	0.023	7.15	0.022	7.25
6 Months	0.026	6.9	0.023	7.15	0.022	7.2	0.019	7.35
9 Months	0.025	6.9	0.023	7.15	0.022	7.2	Separation -	
12 Months	0.025	6.9	0.022	7.2	0.020	7.25	-	-
18 Months	0.024	6.9	0.020	7.2	0.020	7.25	-	-

* All the creams (No. 1 to 27) were subjected to stability study but the data on these selected for blanching test only is

6.1.a. Identification of related foreign steroids.

Thin layer chromatography (TLC) was carried out using silica gel G as the coating substance and a mixture of 77:15:8 of methylene chloride, solvent ether, methyl alcohol and 1.2 volumes of water was taken as mobile phase.

10 µl of each of two solutions was applied to the plate, in a mixture of 9 volumes of chloroform and 1 volume of methyl alcohol containing

(1) 0.15 percent w/v of the corticosteroid being examined

(2) 0.15 percent w/v of the appropriate reference corticosteroid and samples were analysed for presence or absence of related foreign steroids as per the procedure described in IP.[15]

Samples were withdrawn at the end of 1,3,6,9,12 and 18th months and subjected to both physical and chemical examination. All the formulations were assayed as per the methods described in Chapter 2. The observations are recorded in Tables 8-1 to 8-4.

6.2. Microbial Contamination.

Chemical preservatives for semisolids must be carefully evaluated for their stability with regard to the other components of the formulation as well as to the container. Plastic containers may absorb the preservative and thereby decrease the quantity available for inhibiting or destroying the micro-organisms responsible for spoilage

of the product. Some preservatives may sting or irritate the skin.

The preservatives are added to semisolids to prevent contamination, deterioration, and spoilage by bacteria and fungi, since many of the components in these preparations serve as substrates for these microorganisms. Several terms are used to describe microbial organisms associated with pharmaceutical and cosmetic products like "harmful", "objectional" and "opportunistic".

The term "harmful" refer to microbial organisms or their toxins which are responsible for human disease or infection.

An "objectionable" organism can cause disease or its presence may interrupt the function of the drug or lead to the deterioration of the product. Organisms are defined as "opportunistic" pathogens if they produce disease or infection under special environmental situations, as in the new born or the weak person.

The success or failure of the preservative in protecting a formulation against microbial spoilage depends upon many factors. The interaction of the preservative with surfactants, active substances, other components of the vehicle, sorption by polymeric packaging materials, and the product storage temperature may change the concentration of the unbound or free preservative in the aqueous phase.

Topical formulations contain aqueous and oily phases, together with carbohydrates and even proteins, and thus these bases are prone to attack by bacteria and fungi. Microbial growth not only spoils the formulation but is a potential toxicity hazard and a source of infection. Condition which lower immunity, such as bodily injury, debilitating diseases, or drug therapy, may encourage organisms that are usually not highly infectious to infect a host, i.e. to become opportunistic pathogens[16]. Katz[17] observed that in many formulations gram-negative organisms were present which were a health hazard. It was thought that contaminated topical formulations were responsible for hospital infections with gram-negative organisms.[18] It is especially important to preserve topicals which the patient may apply to broken or inflamed skin. The preservative concentration should be lethal to microorganisms rather than simply inhibitory.

The potential sources of microbial contamination are many and varied. Such contamination can occur in raw materials and water used in manufacturing, in processing and filling equipment, in packing materials such as drums, sacks, and cartons and finally containers, if there is an unclean environment or poor plant hygiene, and if plant operatives fail to comply with good manufacturing procedures.[19]

Chemical methods of preservation employ agents which inhibit microbial growth and thus constrain the subsequent

decomposition of product. Ideally, a suitable preservative should not only destroy potential pathogens but should extend the shelf life of the product and minimise the deterious consequences of microbial contamination arising during use of the product.

Preservatives impede microbial metabolism, growth and multiplication by a combination of mechanisms. They may oxidise, reduce, or hydrolyse cellular constituents, act on enzymes or other proteins; interfere with essential metabolites, or modify membrane permeability. Coates[20] reviewed the interaction of preservatives with suspending agents, and Murray and Smith[21] evaluated the incompatibilities of preservatives.

Nonionic and anionic surfactants may be metabolised, whereas cationics inhibit growth. Some surfactants can complex with the preservative below the critical micelle concentration, and the preservative may solubilise within the micelle and so become inactivated. At low concentrations (below the critical micelle concentration) a surfactant may promote a bactericidal action by lowering the interfacial tension at the cell wall.[22,23]

Some workers[7,9-12] reviewed the use of antimicrobial agents in dermatological and cosmetic formulations and mathematical models for calculating the concentration of preservatives available within the aqueous phase of an emulsion.[22, 24-27]

A survey of literature clearly establishes that the topical creams containing corticosteroids are liable to

microbial attack and also keeping in mind the current trends in good manufacturing practices, it was thought worthwhile to subject the selected creams to an evaluation of microbial contamination.

Procedure

6.2.a. Aerobic microbial count

1. Direct transfer procedure :

10 g of cream was dissolved in sterile phosphate buffer (pH 7.2) with 0.1% polysorbate 80. The above mixture was shaken well by keeping flask on a shaker, till it became a suspension. The above mixture was diluted further to yield 30 to 300 colonies per ml. 1 ml of the final diluted mixture was pipetted out in each of three sterile petridishes. Immediately 20 ml of soyabean casein digest agar medium that had been previously been melted and cooled to about 45°, was added to each dish. The petridishes were covered and the samples were mixed with the agar medium by tilting or rotating the dishes and allowed it to solidify at room temperature. The petridishes were inverted and incubated for 48 hrs at 30-32°. After incubation the plates were examined for growth. The number of colonies were counted and expressed the average of three in terms of number of micro-organisms per gm. of the substance.

6.2.b. Gas formers and pathogenic organisms

1. Gas formers.

1 g of cream was inoculated into a 100 ml nutrient broth and shaken well. The above mixture was incubated at 37° for 18 hrs. After incubation 1 ml of enrichment culture was added to a tube containing 10 ml of lactose broth having Durham's tube inside (for detection of gas) and incubated at 37° for 48 hrs. The tubes were examined for acid and gas.

2. Test for Salmonella.

1 g of cream was taken into a nutrient broth medium. The above mixture was shaken well and incubated at 37° for 48 hrs. After incubation the above content was added into a selenite F broth and tetrathionate broth and incubated at 37° for 48 hrs. From each tubes cultures were inoculated in plates containing a layer of brilliant green agar and bismuth sulphite agar. The plates were incubated at 37° for 24 hrs. The plates were examined for presence or absence of colonies.

3. Test for Pseudomonas.

1 g of cream taken into a cetrimide broth medium. The above mixture was shaken well and incubated at 30-32° for 48 hrs and subcultured on a plate containing layer of cetrimide agar and

incubated at 30-32° for 48 hrs. The growth examined by gram staining and the oxidase test was done. The composition of medium used, as per the formulae given in IP[28]. The observations of viable counts are recorded in Tables 8-8 to 8-9.

TABLE 8-5 : Total Viable Count of the Cream Bases.

Cream Base No.	Preservative Concentration	Bacterial Count Total/gm		Gas Formers	Pathogenic Organisums
		Initial	R.T. on storage		
1	A	850	9700	+	-
	B	950	800	+	-
	C	850	550	+	-
2	A	350	450	-	-
	B	280	< 100	-	-
	C	250	< 100	-	-
3	A	5250	110000	-	-
	B	4850	520	-	-
	C	4250	< 100	-	-
4	A	1100	3200	+	-
	B	1050	900	+	-
	C	1050	250	-	-
5	A	800	12000	+	-
	B	950	850	+	-
	C	950	250	-	-
6	A	250	1400	-	-
	B	< 100	< 100	-	-
	C	< 100	< 100	-	-
7	A	2800	120000	+	-
	B	2600	4550	+	-
	C	2550	< 100	-	-

TABLE 8-5 : Contd.

Cream Base No.	Preservative Concentration	Bacterial Count Total/gm		Gas Formers	Pathogenic Organisums
		Initial	R.T. on Storage		
8	A	< 100	350	-	-
	B	< 100	< 100	-	-
	C	< 100	< 100	-	-
9	A	550	35000	-	-
	B	550	450	-	-
	C	500	< 100	-	-
10	A	3050	125000	+	-
	B	2900	550	-	-
	C	3150	< 100	-	-
11	A	250	12000	-	-
	B	200	150	-	-
	C	250	< 100	-	-
12	A	< 100	< 100	-	-
	B	< 100	< 100	-	-
	C	< 100	< 100	-	-
13	A	250	2500	-	-
	B	250	< 100	-	-
	C	200	< 100	-	-
14	A	4550	155000	+	-
	B	4250	3950	+	-
	C	4350	900	+	-
15	A	550	2500	-	-
	B	550	700	-	-
	C	500	250	-	-

TABLE 8-5 : Contd.

Cream Base No.	Preservative Concentration	Bacterial Count Total/gm		Gas Formers	Pathogenic Organisums
		Initial	R.T. on Storage		
16	A	< 100	< 100	-	-
	B	< 100	< 100	-	-
	C	< 100	< 100	-	-
17	A	< 100	300	-	-
	B	< 100	< 100	-	-
	C	< 100	< 100	-	-
18	A	350	1200	-	-
	B	250	< 100	-	-
	C	400	< 100	-	-
19	A	< 100	950	-	-
	B	< 100	< 100	-	-
	C	< 100	< 100	-	-
20	A	< 100	500	-	-
	B	< 100	< 100	-	-
	C	< 100	< 100	-	-
21	A	4200	9000	-	-
	B	4250	3250	-	-
	C	4000	1300	-	-
22	A	450	1300	-	-
	B	250	< 100	-	-
	C	350	< 100	-	-
23	A	< 100	< 100	-	-
	B	< 100	< 100	-	-
	C	< 100	< 100	-	-
24	A	250	1100	-	-
	B	150	< 100	-	-
	C	300	< 100	-	-

TABLE 8-5 : Contd.

Cream Base No.	Preservative Concentration	Bacterial Count Total/gm		Gas Formers	Pathogenic Organisums
		Initial	R. T. on storage		
25	A	650	10,000	-	-
	B	550	1,000	-	-
	C	550	450	-	-
26	A	< 100	< 100	-	-
	B	< 100	< 100	-	-
	C	< 100	< 100	-	-
27	A	< 100	< 100	-	-
	B	< 100	< 100	-	-
	C	< 100	750	-	-

\+ Detected

\- Not Detected

A Methyl Paraben Sodium - 0.18%
Propyl Paraben Sodium - 0.02%

B Methyl Paraben Sodium - 0.3%
Propyl Paraben Sodium - 0.05%

C Chlorocresol - 0.1%

TABLE 8-6 : Total Viable Count during Stability of Triamcinolone Acetonide Creams*

Cream No.	Preservative Concentration	Total Bacterial Count				
		Initial	3M	6M	9M	12M
1	0.1% (C)	700	500	350	200	< 100
5	0.1% (C)	1200	< 100	< 100	< 100	< 100
13	0.3% (A) + 0.05% (B)	250	< 100	< 100	< 100	< 100
25	0.1% (C)	450	450	350	350	250
27	0.18% (A) + 0.02% (B)	< 100	< 100	< 100	< 100	< 100

Key A - Methyl paraben sodium

B - Propyl paraben sodium

C - Chlorocresol

* All the selected TA creams were subjected to total viable count study at R.T. (29 ± 2°), but the data of only the selected for the blanching test is tabulated.

TABLE 8-7 : Total Viable Count during Stability of Betamethasone 17-Valerate Creams*

Cream No.	Preservative Concentrations	Total Bacterial Count				
		Initial	3M	6M	9M	12M
1	0.1% (C)	800	750	300	200	< 100
5	0.1% (C)	1050	850	250	< 100	< 100
7	0.1% (C)	1800	900	550	450	450
13	0.3% (A) + 0.05% (B)	< 100	< 100	< 100	< 100	< 100
27	0.18% (A) + 0.02% (B)	< 100	< 100	< 100	< 100	< 100

Key A - Methyl paraben sodium

B - Propyl paraben sodium

C - Chlorocresol.

* All the selected BV creams were subjected to total viable count study at R.T. (29 ± 2°) but the data of only the selected for the blanching test is tabulated.

TABLE 8-8 : Total Viable Count during Stability of Halcinonide Creams*

Cream No.	Preservative Concentration	Total Bacterial Count				
		Initial	3M	6M	9M	12M
1	0.1% (C)	950	350	350	<100	<100
5	0.1% (C)	850	<100	<100	<100	<100
13	0.3% (A) 0.05% (B)	<100	<100	<100	<100	<100
25	0.1% (C)	600	450	450	300	150
27	0.18% (A) 0.02% (B)	<100	<100	<100	<100	<100

Key A - Methyl Paraben sodium

B - Propyl paraben sodium

C - Chlorocresol

* All the selected HAL Creams were subjected to total viable count study at R.T. (29 ± 2°), but the data of only the selected for the blanching test is tabulated.

TABLE 8-9 : Total Viable Count during Stability of Fluocinolone Acetonide Creams*

Cream No.	Preservative Concentration	Total Bacterial Count				
		Initial	3M	6M	9M	12M
1	0.1% (C)	1100	450	250	< 100	< 100
5	0.1% (C)	950	350	< 100	< 100	< 100
13	0.3% (A) 0.05% (B)	< 100	< 100	< 100	< 100	< 100
25	0.1% (C)	350	250	250	150	< 100
27	0.18% (A) 0.02% (B)	< 100	< 100	< 100	< 100	< 100

Key A - Methyl paraben sodium

B - Propyl paraben sodium

C - Chlorocresol.

* All the selected FA Creams were subjected to total viable count study at R.T. (29 ± 2°), but the data of only the selected for the blanching test is tabulated.

6.3. Results and Discussion

Selected corticosteroid creams were kept on stability at different conditions for one and half year. The observations are recorded in Tables 8-1 to 8-4.

(a) Triamcinolone acetonide creams

It is clearly observed from the data recorded in Table 8-1 that cream Nos., 1,5, 13 and 27 are stable in all conditions. The degradation products were absent in all above formulations. In case of cream No. 25 separation was observed after nine months at 42°/ 80% R.H., and after one and half year at 37°.

(b) Betamethasone 17-valerate creams

It is clearly observed from the data recorded in Table 8-2 that cream Nos. 1, 13 and 27 are stable in all conditions. Cream No. 7 separated after three months of storage at 42°/80% R.H. and cream No. 5 separated at 42°/80% R.H. at the end of one and half year stability. The degradation products were absent in all above formulations.

(c) Halciponide creams

It is clearly observed from the data recorded in Table 8-3 that cream Nos. 1,5,13, 25 and 27 are stable in all conditions during storage of one and half year. The degradation products were absent in all the above mentioned

formulations.

(d) **Fluocinolone acetonide creams**

It is clearly observed from the data recorded in Table 8-4 that cream No. 5 is stable in all the conditions during storage of one and half year. Cream Nos. 1 and 25 were separated at the end of one and half year at 42°/80% R.H. Cream No. 13 was separated after six months at 42°/80% R.H. and cream No. 27 was separated after nine months at 42°/80% R.H. Cream No. 13 showed degradation of FA from 0.025% to 0.02% after one and half year. In case of cream No. 27 the degradation was observed and the FA concentration was reduced from 0.025% to 0.019% after six months of storage at 42°/80% R.H. Cream No. 25 also showed degradation of FA from 0.026% to 0.021% after twelve months at 42°/80% R.H. However, no degradation was observed at A.C. temperature.

The characteristics like physical stability, separation, spreadability, washability, consistancy, pH, water retention, congealing points, compatibility were checked before and during stability and all the promising creams were found to be satisfactory at A.C. and R.T. during one and half year of stability.

Selected cream bases were prepared with different concentrations of methyl paraben sodium - propyl paraben sodium combination and chlorocresol as a preservatives. After stability of one month at R.T. (29°±1°), samples were taken and analysed for total viable count and presence or absence of gas formers. If the gas formers were present then those samples were checked for presence of pathogenic organisms.

It is clearly observed from the data recorded in Table 8-5 that cream base Nos. 2,3,6,8,9,11,12,13 and 15 to 27 show no detection of gas formers or pathogenic organisms, even though cream base Nos. 3,9,11,13,15,21, 22,24 and 25 show very high viable counts at lower concentration of combinations of methyl paraben sodium (0.18%)-propyl paraben sodium (0.02%).

It is also observed from the data recorded in Table 8-5 that cream base Nos. 1,4,5,7,10 and 14 show high bacterial counts with methyl paraben sodium propyl paraben sodium combinations. In all the above six cream bases gas formers were detected and they were checked for pathogenic organisms, pathogens were absent.

The bacterial counts dropped to a greater extent in the selected cream bases containing 0.1% chlorocresol as a preservative. In case of cream Nos. 7 and 10, the bacterial count dropped to less than 100 colonies/g and in case of cream base Nos. 4 and 5, the bacterial count dropped to 250 colonies/g from 1050 colonies/g and

950 colonies/g respectively and no gas formation was observed in above four cream bases as it was observed with methyl paraben sodium-propyl paraben sodium combinations.

After comparing the data of viable counts of all the selected cream bases, fresh creams were prepared with suitable preservatives and antioxidants like butylated hydroxy anisole(0.01%) and butylated hydroxy toluene (0.01%) in combinations along with a sequestering agent disodium edetate (0.2%) were used in cream Nos. 1 and 14 as cream base Nos. 1 and 14 showed more viable counts and presence of gas formers. All the above prepared creams were kept for stability at R.T. (29±2°).

It is observed from the data recorded in Tables 8-6 to 8-9 that the suitable preservatives used in selected creams are bactericidal and in all the selected creams the bacterial counts reduce during storage. The gas formers were absent in all the selected creams.

Chlorocresol was found superior to the methyl paraben sodium - propyl paraben sodium combinations, probably because of the increase in phenolic strength imparted to the hydroxy-group by halogen atoms ortho- or para- to it.

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